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L8: Entry 3 of 7 File: PGPB Dec 4, 2003

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DOCUMENT-IDENTIFIER: US 20030225016 A1

TITLE: Chimeric immunomodulatory compounds and methods of using the same - III

PUBLICATION-DATE: December 4, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
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Dina, Dino	Oakland	CA	US	
Tuck, Stephen F.	Oakland	CA	US	

US-CL-CURRENT: 514/44; 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 4. Document ID: US 20030199466 A1

L8: Entry 4 of 7 File: PGPB Oct 23, 2003

PGPUB-DOCUMENT-NUMBER: 20030199466
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030199466 A1

TITLE: Chimeric immunomodulatory compounds and methods of using the same - II

PUBLICATION-DATE: October 23, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fearon, Karen L.	Lafayette	CA	US	
Dina, Dino	Oakland	CA	US	
Tuck, Stephen F.	Oakland	CA	US	

US-CL-CURRENT: 514/44; 525/54.2, 536/23.1

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

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L8: Entry 5 of 7 File: PGPB Sep 18, 2003

PGPUB-DOCUMENT-NUMBER: 20030175731
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DOCUMENT-IDENTIFIER: US 20030175731 A1

TITLE: Chimeric immunomodulatory compounds and methods of using the same - I

PUBLICATION-DATE: September 18, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fearon, Karen L.	Lafayette	CA	US	
Dina, Dino	Oakland	CA	US	
Tuck, Stephen F.	Oakland	CA	US	

US-CL-CURRENT: 435/6; 514/44, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 6. Document ID: US 20030049266 A1

L8: Entry 6 of 7 File: PGPB Mar 13, 2003

PGPUB-DOCUMENT-NUMBER: 20030049266
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20030049266 A1

TITLE: Immunomodulatory polynucleotides and methods of using the same

PUBLICATION-DATE: March 13, 2003

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fearon, Karen L.	Lafayette	CA	US	
Dina, Dino	Oakland	CA	US	

US-CL-CURRENT: 424/185.1; 435/320.1, 435/325, 435/69.3, 514/44, 530/350, 536/23.2

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 7. Document ID: AU 2003297483 A1, WO 2004058179 A2

L8: Entry 7 of 7 File: DWPI Jul 22, 2004

DERWENT-ACC-NO: 2004-525782
DERWENT-WEEK: 200476

TITLE: Immunomodulatory polynucleotide useful for the treatment of e.g. atopic dermatitis comprises palindromic sequence comprising at least eight bases in length, which contains at least two dinucleotides and at least one trinucleotide

INVENTOR: DINA, D; FEARON, K L ; MARSHALL, J

PRIORITY-DATA: 2003US-467546P (May 1, 2003), 2002US-436122P (December 23, 2002), 2003US-447885P (February 13, 2003)

PATENT-FAMILY:

PUB-NO	PUB-DATE	LANGUAGE	PAGES	MAIN-IPC
AU 2003297483 A1	July 22, 2004		000	A61K000/00
WO 2004058179 A2	July 15, 2004	E	119	A61K000/00

INT-CL (IPC): A61 K 0/00

ABSTRACTED-PUB-NO: WO2004058179A
BASIC-ABSTRACT:

NOVELTY - An immunomodulatory polynucleotide (IMP), comprises: a palindromic sequence comprising at least 8 bases in length, which contains at least two dinucleotides, and at least one trinucleotide at or near the 5' end of the polynucleotide.

DETAILED DESCRIPTION - An immunomodulatory polynucleotide, comprises: a palindromic sequence at least 8 bases in length which contains at least two CG dinucleotides, and at least one trinucleotide of formula (TCG)y at or near the 5' end of the polynucleotide. The CG dinucleotides are separated by 0 - 5 bases. The 5' T of the (TCG)y is positioned 0 - 3 bases from the 5' end of the polynucleotide. The (TCG)y is separated from the 5' end of the palindromic sequence by 0 - 2 bases. The palindromic sequence includes all or part of the (TCG)y sequence.

y = 1 or 2.

ACTIVITY - Antimicrobial; Antiallergic; Antiasthmatic; Dermatological; Antiinflammatory; Ophthalmological; Immunosuppressive; Antibacterial; Vasotropic; Antiparasitic; Virucide; Hepatotropic; Anti-HIV; Cytostatic; Antiulcer; Gastrointestinal-Gen.; Nephrotropic.

MECHANISM OF ACTION - Immune response modulator; Cytokine (type I interferons e.g. interferon (IFN)- alpha and IFN- omega and IFN- gamma) stimulator; Activator of plasmacytoid dendritic cells to undergo maturation; Vaccine. The immunomodulatory polypeptide (IMP) was tested for stimulation of improved natural killer (NK) cell lytic activity. Peripheral blood mononuclear cells (PBMCs) were stimulated with immunomodulatory polypeptide (IMP) (having sequence of formula 5'-TCGTCGAACGTTCGAGATGAT) (10 mg/ml) or negative control polynucleotide for 48 hours in culture. The treated PBMCs were then co-cultured with 51Cr-loaded K562 tumor target cells for 4 hours. 51Cr released upon cell lysis was measured. It was found that IPM with palindromes of 12 bases in length stimulated an increased amount of NK cell lytic activity.

USE - For ameliorating a symptom of an infectious disease and IgE-related disorder (claimed). For the treatment of a disorder associated with a T helper (TH)2-type immune response (e.g. allergies, allergy-induced asthma or atopic dermatitis), individuals receiving vaccines such as therapeutic vaccines (e.g. vaccines comprising an allergy epitope, a mycobacterial epitope or a tumor associated epitope) or prophylactic vaccines. Also for the treatment of e.g. food allergies, rhinitis, atopic dermatitis, conjunctivitis, urticaria, shock, Hymenoptera sting allergies and drug allergies and parasitic infections; viral disease e.g. hepatitis B, hepatitis C, influenza, acquired immunodeficiency syndrome (AIDS) and herpes zoster; and cancer; inflammatory disorder e.g. ulcerative colitis; fibrotic disorder e.g. idiopathic pulmonary fibrosis, scleroderma, cutaneous radiation-induced fibrosis, hepatic fibrosis including schistosomiasis-induced hepatic fibrosis, renal fibrosis. As a prophylactic vaccine to increase resistance to

infection by bacterial or viral pathogens.

ADVANTAGE - The immunomodulatory polynucleotide modulates an immune response; or increases interferon- gamma or interferon- alpha ; effectively stimulates cytokine including type I interferons e.g. IFN- alpha and IFN- omega and IFN- gamma , production from human cells; effectively stimulates B cells to proliferate; activates plasmacytoid dendritic cells to undergo maturation; can result in retardation of plasmacytoid dendritic cell apoptosis in culture.

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Terms	Documents
(L7 OR L6) AND L5	7

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Search Results - Record(s) 1 through 7 of 7 returned.

☐ 1. Document ID: US 20040136948 A1

L8: Entry 1 of 7 File: PGPB Jul 15, 2004

PGPUB-DOCUMENT-NUMBER: 20040136948
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040136948 A1

TITLE: Branched immunomodulatory compounds and methods of using the same

PUBLICATION-DATE: July 15, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fearon, Karen L.	Lafayette	CA	US	

US-CL-CURRENT: 424/78.29; 514/44

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

☐ 2. Document ID: US 20040132677 A1

L8: Entry 2 of 7 File: PGPB Jul 8, 2004

PGPUB-DOCUMENT-NUMBER: 20040132677
PGPUB-FILING-TYPE: new
DOCUMENT-IDENTIFIER: US 20040132677 A1

TITLE: Chimeric immunomodulatory compounds and methods of using the same-IV

PUBLICATION-DATE: July 8, 2004

INVENTOR-INFORMATION:

NAME	CITY	STATE	COUNTRY	RULE-47
Fearon, Karen L.	Lafayette	CA	US	
Dina, Dino	Oakland	CA	US	
Tuck, Stephen F.	Oakland	CA	US	

US-CL-CURRENT: 514/44; 514/7, 514/8, 536/23.5

Full | Title | Citation | Front | Review | Classification | Date | Reference | Sequences | Attachments | Claims | KWIC | Draw D

L17: Entry 6 of 411 File: PGPB Jun 2, 2005

DOCUMENT-IDENTIFIER: US 20050119273 A1
TITLE: Small molecule toll-like receptor (TLR) antagonists

Detail Description Paragraph:
[0233] In contrast, with respect to dendritic cells (DC), Mazzoni and others have reported that histamine inhibits IL-12 production and stimulates IL-10 secretion in LPS-stimulated monocyte-derived DC, resulting in a shift from a Th1- to a Th2-polarized immune response. Mazzoni A et al. (2001) J Clin Invest 108:1865; Caron G et al. (2001) J Immunol 166:6000-6. Recently Mazzoni et al. also reported their observation that histamine, acting through H2R, inhibits release of type I IFN and TNF-.alpha. by plasmacytoid DC (pDC), the principal producers of IFN-.alpha., in response to exposure to CpG ODN or live influenza virus. Mazzoni A et al. (2003) J Immunol 170:2269-73. Finally, it was recently reported that histamine, acting through H2R on monocyte/macrophages, suppresses NADPH oxidase, a key enzyme in oxygen radical formation, resulting in protection of natural killer (NK) cells and T cells against oxygen radical-induced dysfunction and apoptosis. Hellstrand K (2002) Semin Oncol 29(3 Suppl 7):35-40.

Detail Description Paragraph:
[0245] CpG ODN have been further classified by structure and function into at least the following three classes or types, all of which are intended to be encompassed within the term CpG DNA as used herein: B-class CpG ODN such as ODN 2006 include the originally described immunostimulatory CpG ODN and characteristically activate B cells and NK cells but do not induce or only weakly induce expression of type I interferon (e.g., IFN-.alpha.). A-class CpG ODN, described in published PCT international application WO 01/22990, incorporate a CpG motif, include a chimeric phosphodiester/phosphorothioate backbone, and characteristically activate NK cells and induce plasmacytoid dendritic cells to express large amounts of IFN-.alpha. but do not activate or only weakly activate B cells. An example of an A-class CpG ODN is 5'-G*G*G_G_G_A_C_G_A_T_C_G_T_C_G*G*G*G*G- *G-3' (ODN 2216, SEQ ID NO:2), wherein "*" represents phosphorothioate and "_" represents phosphodiester. C-class CpG ODN incorporate a CpG, include a wholly phosphorothioate backbone, include a GC-rich palindromic or nearly-palindromic region, and are capable of both activating B cells and inducing expression of IFN-.alpha.. C-class CpG ODN have been described, for example, in published U.S. patent application 2003/0148976. An example of a C-class CpG ODN is 5'-TCGTCGTTTTCGGCGCGCGCCG-3' (ODN 2395; SEQ ID NO:3). For a review of the various classes of CpG ODN, see also Vollmer J et al. (2004) Eur J Immunol 34:251-62.

Detail Description Paragraph:
[0296] As used herein, the terms "CpG nucleic acid" and, equivalently, "CpG ODN" refer to an immunostimulatory nucleic acid which contains a cytosine-guanine (CG) dinucleotide, the C residue of which is unmethylated. The effects of CpG nucleic acids on immune modulation have been described extensively in U.S. patents such as U.S. Pat. Nos. 6,194,388; 6,207,646; 6,218,371; and 6,239,116, and published international patent applications, such as WO 98/37919, WO 98/40100, WO 98/52581, and WO 99/56755. The entire contents of each of these patents and published patent applications is hereby incorporated by reference. The entire immunostimulatory nucleic acid can be unmethylated or portions may be unmethylated but at least the C of the 5'-CG-3' must be unmethylated. The CpG nucleic acid sequences of the

invention include those broadly described above as well as disclosed in U.S. Pat. Nos. 6,207,646 B1 and 6,239,116 B1.

Detail Description Paragraph:
[0298] CpG nucleic acids have been further classified by structure and function into at least the following three types, all of which are intended to be encompassed within the methods of the instant invention: Type B CpG nucleic acids such as ODN 2006 include the earliest described CpG nucleic acids and characteristically activate B cells but do not induce or only weakly induce expression of IFN-.alpha.. Type A CpG nucleic acids, described in published international application PCT/US00/26527 (WO 01/22990), incorporate a CpG motif, include a hybrid phosphodiester/phosphorothioate backbone, and characteristically induce plasmacytoid dendritic cells to express large amounts of IFN-.alpha. but do not activate or only weakly activate B cells. Type C oligonucleotides incorporate a CpG, include a chimeric backbone, include a GC-rich palindromic or nearly-palindromic region, and are capable of both activating B cells and inducing expression of IFN-.alpha.. These have been described, for example, in published U.S. patent application 2003/0148976.

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L19: Entry 5 of 192 File: PGPB Oct 31, 2002

DOCUMENT-IDENTIFIER: US 20020160038 A1
TITLE: Liposomes containing oligonucleotides

Pre-Grant Publication Year:
2002

Detail Description Paragraph:
[0042] raff As-ODNs is a biologic radiosensitizer of SQ-20B cells Radiation survival dose responses of SQ-20B cells exposed to S-and As-ODNs (100 pmol/.mu.l, 12 hr) were evaluated. (S- and As-ODNs used in this study do not contain the G-quartet or CpG motifs previously shown to be responsible for non-antisense-specific effects such as enhanced affinity for protein or interference with the immune response.) The plating efficiencies indicated that the As-ODNs treatment had no effect on cell viability as compared to S-ODNs-treated cells (Table 1). These data are also in agreement with the S- and As-ODNs effects on the viability of logarithmically growing cells discussed earlier. Radiation survival dose responses of the control (without oligo) and S-ODNs-treated cells were almost identical. Most important, As-ODNs treatment resulted in decreases of the shoulder and the slope of the survival curve. The radiobiological parameters were obtained by fitting the data (surviving number of colonies) to the single-hit multitarget (D, D.sub.q, n) and linear-quadratic (.alpha., .beta.) models of radiation survival response. In addition, the value of a model-free parameter, mean inactivation dose (D) was calculated (14) (Table 1). Based on a ratio of the mean inactivation dose, the dose modifying factor (DMF) of As-ODNs treatment was .about.1.4. Significant decreases observed in the values of radiobiological parameters, D, D.sub.q, and D.sub.0 of SQ-20B cells following treatment with the raf As-ODNs indicate a good correlation between the DNA sequence-specific inhibition of Raf-1 protein kinase and the radiosensitization of these relatively radioresistant tumor cells.

L19: Entry 8 of 192 File: PGPB Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020142978 A1
TITLE: Synergistic improvements to polynucleotide vaccines

Abstract Paragraph:
The invention features a polynucleotide vaccine modified to enhance expression of the encoded antigen in host cells. The polynucleotide vaccine comprises an antigen-encoding nucleic acid sequence derived from a non-host species of a first phylum or first kingdom, wherein the native signal sequence of the antigen coding sequence is deleted and, optionally, replaced with a signal sequence of a polypeptide of a second phylum or a second kingdom that is functional in the host to be immunized (e.g., a viral signal sequence with a plant antigen-encoding sequence). In one embodiment, the signal sequence is a hemagglutinin A (HA) signal sequence, and the antigen is an allergen (e.g., plant allergen) or from a pathogen (e.g., a bacterium, virus or parasite). The polynucleotide vaccine of the invention provides a synergistic effect with an immunostimulatory sequence (ISS) adjuvant to not only maintain, but to enhance, the immune response to the encoded antigen.

Pre-Grant Publication Year:
2002

Summary of Invention Paragraph:
[0005] Immunostimulatory DNA sequences (ISS) delivered in conjunction with an antigen activate innate immunity and bias the adaptive immune response toward Th1 differentiation, thus shifting the immune response away from a Th2 response, which includes immune responses associated with allergy. ISS have been used as an adjuvant to amplify the immune response to a co-delivered antigen. See, for example, WO 98/16247, and U.S. Pat. Nos. 5,736,524 and 5,780,448. The use of ISS, particularly at a high dose (e.g., greater than 10 .mu.g), as an adjuvant with gene vaccines, however, has resulted in reduced antigen expression and failure to elicit immunostimulatory effects (Weeratna et al., 1998, Antisense & Nucleic Acid Drug Development 8:351-356). Thus, ISS have been considered useful with DNA vaccines only if the ISS is positioned within the DNA vaccine itself, either endogenously or through subcloning (Krieg et al., 1998, Trends Microbiol. 6(1):23-7; Weeratna et al., supra).

Summary of Invention Paragraph:
[0007] The invention features a polynucleotide vaccine modified to enhance expression of the encoded antigen in host cells. The polynucleotide vaccine comprises an antigen-encoding nucleic acid sequence derived from a non-host species of a first phylum or first kingdom, wherein the native signal sequence of the antigen coding sequence is deleted and, optionally, replaced with a signal sequence of a polypeptide of a second phylum or a second kingdom that is functional in the host to be immunized (e.g., a viral signal sequence with a plant antigen-encoding sequence). In one embodiment, the signal sequence is a hemagglutinin A (HA) signal sequence, and the antigen is an allergen (e.g., plant allergen) or from a pathogen (e.g., a bacterium, virus or parasite). The polynucleotide vaccine of the invention provides a synergistic effect with an immunostimulatory sequence (ISS) adjuvant to not only maintain, but to enhance, the immune response to the encoded antigen.

Summary of Invention Paragraph:

[0010] The invention additionally provides a method for modulating an immune response to an antigen, and a method for eliciting an immune response to an antigen. The method comprises administering to a subject a polynucleotide vaccine of the invention. The method preferably further comprises administering to the subject an immunostimulatory nucleotide sequence (ISS). Administration of both the polynucleotide vaccine and the ISS achieves a synergistic improvement in efficacy of the method. In one embodiment, the antigen is an allergen, such as a grass pollen or ragweed, latex, cat dander, food (such as peanut), house dust mite or cockroach allergen.

Detail Description Paragraph:
[0036] The terms "immunomodulatory nucleic acid molecule," "immunostimulatory nucleic acid molecule," "immunostimulatory oligonucleotide sequence," "immunostimulatory polynucleotide sequence," "immunomodulatory polynucleotide sequence," "ISS," "ISS-PN," and "ISS-ODN," are used interchangeably herein to refer to a polynucleotide that comprises at least one immunomodulatory nucleic acid moiety. "ISS" is often used for ease of reference and clarity, but is not meant to be limiting. The terms "immunomodulatory," and "immunostimulatory," as used herein in reference to a nucleic acid molecule, refer to the ability of a nucleic acid molecule to modulate an immune response in a vertebrate host. In particular, these terms refer to the ability of an immunostimulatory nucleic acid molecule to increase an immune response in a vertebrate host, particularly to increase a CTL response, particularly an antigen-specific CTL response. Such nucleic acid molecules have at least one ISS moiety.

Detail Description Paragraph:
[0059] Immunomodulatory nucleic acid molecules are polynucleotides that modulate activity of immune cells, especially immune cell activity associated with a type-1 (Th1-mediated) or type-1 like immune response. Furthermore, immunomodulatory nucleic acid molecules of the present invention encompass polynucleotides that modulate an immune response to an antigen so as to provide for protection against subsequent exposure to the antigen, e.g., in the context of vaccination against a pathogen or in the context of allergic immunotherapy. The immunomodulatory nucleic acid (often referred to herein for convenience as ISS) useful in the invention includes an oligonucleotide, which can be a part of a larger nucleotide construct such as a plasmid.

Detail Description Paragraph:
[0088] Confirmation that a particular compound has the properties of an immunomodulatory nucleic acid molecule useful in the invention can be obtained by evaluating whether the immunomodulatory nucleic acid molecule elicits the appropriate cytokine secretion patterns, e.g., a cytokine secretion pattern associated with a type-1 immune response. ISS delivered with an antigen also induces activity of cytotoxic T cells and acts as a very strong mucosal adjuvant (see, e.g., Horner (1998) Cell. Immunol. 190:77-82). As noted above, immunomodulatory nucleic acid molecules of interest in the methods of the invention are those that elicit a Th1 -mediated response, and/or, where the antigen is an allergen, shift the immune response away from an allergic immune response.

Detail Description Paragraph:
[0121] The polynucleotide vaccines of the invention are administered to an individual using any available method and route suitable for drug delivery, including systemic, mucosal, and localized routes of administration. In a preferred embodiment of the method, the polynucleotide vaccine is administered via a systemic, enteral or topical route. Examples of systemic routes include, but are not limited to, intradermal, intramuscular, subcutaneous and intravenous administration. Examples of topical routes include, but are not limited to, intranasal, intravaginal, intrarectal, intratracheal, transdermal and ophthalmic administration. Examples of enteral routes include, but are not limited to, oral and gastric administration. Routes of administration may be combined, if desired,

or adjusted depending upon the construct, the number of ISS, the desired effect on the immune response, and other variables that will be readily apparent to the ordinarily skilled artisan. In general, the immunomodulatory nucleic acid can be administered as part of the polynucleotide vaccine (e.g., within the same nucleic acid molecule), or as a separate nucleic acid molecule that is co-administered or separately administered.

Detail Description Paragraph:
[0135] It should be noted that the immunotherapeutic activity of immunomodulatory nucleic acid molecules, as well as the polynucleotide vaccines, is essentially dose-dependent. Therefore, to increase ISS potency by a magnitude of two, each single dose is doubled in concentration. Increased dosages may be needed to achieve the desired therapeutic goal. The invention thus contemplates administration of "booster" doses to provide and maintain an immune response effective to, for example, protect the subject from infection or to inhibit infection; to reduce the risk of the onset of disease or the severity of disease symptoms that may occur as a result of infection; to facilitate reduction of pathogen load; to facilitate clearance of infecting pathogen from the subject (e.g., to facilitate clearance of organisms from the lungs), and the like; or to maintain the resistance of the subject to exposure to allergen (e.g., to protect the subject from a hypersensitivity reaction, e.g., an early or late phase allergic response, including anaphylaxis).

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L19: Entry 9 of 192 File: PGPB Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020142977 A1
TITLE: Methods for increasing a cytotoxic T lymphocyte response in vivo

Pre-Grant Publication Year:
2002

Detail Description Paragraph:
[0054] The terms "immunomodulatory nucleic acid molecule," "immunostimulatory nucleic acid molecule," "ISS," "ISS-PN," and "ISS-ODN," are used interchangeably herein to refer to a polynucleotide that comprises at least one immunomodulatory nucleic acid moiety. The terms "immunomodulatory," and "immunostimulatory," as used herein in reference to a nucleic acid molecule, refer to the ability of a nucleic acid molecule to modulate an immune response in a vertebrate host. In particular, these terms refer to the ability of an immunostimulatory nucleic acid molecule to increase an immune response in a vertebrate host, particularly to increase a CTL response, particularly an antigen-specific CTL response.

Detail Description Paragraph:
[0107] Antigen may be administered separately from the immunostimulatory nucleic acid molecule, in admixture with immunostimulatory nucleic acid molecule, or the immunostimulatory nucleic acid and antigen can be proximately associated with (e.g., conjugated or brought into spatial proximation by other means, as described in more detail below) to one or more immunostimulatory nucleic acid molecules. Generally, and most preferably, an immunomodulatory nucleic acid and an antigen are proximately associated at a distance effective to enhance the immune response generated compared to the administration of the ISS and antigen as an admixture. For a detailed discussion of method for proximate association of a polynucleotide and an antigen see, e.g., PCT Publication WO 00/21556, incorporated herein by reference.

Detail Description Paragraph:
[0143] Immunostimulatory nucleic acid molecule may be administered separately from antigen, in admixture with antigen, or the immunostimulatory nucleic acid can be proximately associated with (e.g., conjugated or brought into spatial proximation by other means, as described in more detail below) one or more antigens (or the antigen can be proximately associated with one or more immunostimulatory nucleic acid molecules). Generally, and most preferably, an immunomodulatory nucleic acid and an antigen are proximately associated at a distance effective to enhance the immune response generated compared to the administration of the ISS and antigen as an admixture. For a detailed discussion of method for proximate association of a polynucleotide and an antigen see, e.g., PCT Publication WO 00/21556, incorporated herein by reference.

L19: Entry 10 of 192 File: PGPB Oct 3, 2002

DOCUMENT-IDENTIFIER: US 20020142974 A1
TITLE: IMMUNE ACTIVATION BY DOUBLE-STRANDED POLYNUCLEOTIDES

Pre-Grant Publication Year:
2002

Summary of Invention Paragraph:
[0046] Bacterial, but not mammalian DNA, can boost the lytic activity of NK cells and induce .gamma.IFN production, an effect attributed to palindromic sequences present in bacterial DNA (S. Yammamoto, el al., J. Immunol. 148: 4072-4076 (1992)). In addition, other investigators showed that bacterial DNA, especially when complexed to DNA-binding proteins, could induce B cell activation. To better define the size and composition of the relevant immunostimulatory motif(s), Krieg and colleagues examined the activity of a series of synthetic oligodeoxynucleotides (ODNs) (A. M. Krieg, et al., Nature 374: 546-548 (1995)). Optimal stimulation was observed when the ODN contained at least one non-methylated CpG dinucleotide flanked by two 5' purines (optimally GpA) and two 3' pyrimidines (optimally TpC or TpT). Immune stimulation persisted despite purine/purine or pyrimidine/pyrimidine replacements, even if these substitutions eliminated a palindromic sequence. Yet if either base pair of the CpG was eliminated, stimulatory activity was lost. Optimizing the flanking region or incorporating two CPGs into a single ODN increased stimulation. The minimal length of a stimulatory ODN was 8 bp. These findings established that immune stimulation was mediated by a six base pair nucleotide motif consisting of an unmethylated CpG dinucleotide flanked by two 5' purines and two 3' pyrimidines imbedded in a larger fragment of DNA (A. M. Krieg, et al., Nature 374: 546-548 (1995)). Such motifs are expressed nearly 20 times more frequently in bacterial than vertebrate DNA due to differences in the frequency of utilization and methylation pattern of CpG dinucleotides in prokaryotes versus eukaryotes.

Summary of Invention Paragraph:
[0047] Evidence suggests that these motifs act directly on cells of the immune system. Cells responsive to CpG ODN include macrophages, B lymphocytes, T lymphocytes, and NK cells. CpG ODN rapidly stimulate B cells to produce IL-6 and IL-12, CD4+ T cells to produce IL-6 and .gamma.IFN, and NK cells to produce .gamma.IFN both in vivo and in vitro (D. M. Klinman, et al., Proc. Natl. Acad. Sci. U.S.A. 93: 2879-2883 (1996)). This lymphocyte stimulation is polyclonal and antigen non-specific in nature, although specificity is retained with respect to the phenotype of cells activated and the type of cytokine they produced. The finding that NK and T cells as well as B cells are triggered by CpG-containing ODNs suggests that immune recognition of this motif is evolutionarily conserved among multiple types of immunologically active cells. Kinetic studies reveal that CpG ODNs induce cytokine release within four hours of administration, with peak production occurring by 12 hours (D. M. Klinman, et al., Proc. Natl. Acad. Sci. U.S.A. 93: 2879-2883 (1996)). Maximal cytokine production is observed using ODNs at a concentration of 0.10-0.33 ug/ml (D. M. Klinman, et al., Proc. Natl. Acad. Sci. U.S.A. 93: 2879-2883 (1996)). Synthetic ODN expressing stimulatory CpG motifs have been used as adjuvants to boost the immune response to DNA and protein based immunogens. In vivo experiments demonstrate that CpG-containing oligos augment antigen-specific antibody production by up to ten fold, and .gamma.IFN production

by up to six fold. For example, CpG ODN boost antigen-specific immune responses when co-administered with either protein- or DNA-based vaccines (Y. M. Sato, et al., Science 273: 352-354 (1996); M. E. Roman, et al., Nature Medicine 3: 849-854 (1997); D. M. Klinman, et al., J. Immunol. 158: 3635-3642 (1997)). This activity is present whether the motifs are intrinsic parts of the antigen (as in the backbone of a DNA vaccine), or co-administered along with the antigen (M. E. Roman, et al., Nature Medicine 3: 849-854 (1997)). However, immunogenicity is improved when the CpG oligo is physically linked to the relevant antigen. This is true both in the case of DNA vaccines and protein antigens. These results confirm the intuitive expectation that optimal stimulation occurs when antigen and adjuvant are presented to the immune system in close spatial and temporal sequence. These data suggest that CpG oligos initiate a complex cascade of events in vivo that may have broad application for immune regulation.

Summary of Invention Paragraph:
[0089] The invention can be distinguished from the effects of CpG sequences because methylation does not alter activity whereas methylation eliminates CpG activity. There is no sequence specificity, whereas optimal CpG stimulation depends on sequence, e.g., when the ODN contains at least one non-methylated CpG dinucleotide flanked by two 5' purines (optimally GpA) and two 3' pyrimidines (optimally TpC or TpT). Most importantly, CpG motifs act directly only on cells of the immune system, whereas the ds nucleic acids described herein also work on nonimmune cells and convert them to APC.

Summary of Invention Paragraph:
[0090] The present invention may be used additively or synergistically with synthetic ODN expressing stimulatory CpG motifs, for example as adjuvants to boost the immune response to DNA and protein based immunogens and when coadministered with protein or DNA-based vaccines (Y. M. Sato, et al., Science 273: 352 (1996); M. E. Roman, et al., Nature Medicine 3: 849 (1997); D. M. Klinman, et al., J. Immunol. 158: 3635 (1997)). The one agent (ds nucleic acids) acts on the nonimmune cells to improve immune recognition; the other (CpG motifs) work on the immune cells to activate their responsiveness.

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L19: Entry 12 of 192 File: PGPB Aug 8, 2002

DOCUMENT-IDENTIFIER: US 20020107212 A1
TITLE: Methods of reducing papillomavirus infection using immunomodulatory polynucleotide sequences

Pre-Grant Publication Year:
2002

Summary of Invention Paragraph:
[0012] Administration of certain DNA sequences, generally known as immunostimulatory sequences or "ISS," induces an immune response with a Th1-type bias as indicated by secretion of Th1-associated cytokines. The Th1 subset of helper cells is responsible for classical cell-mediated functions such as delayed-type hypersensitivity and activation of cytotoxic T lymphocytes (CTLs), whereas the Th2 subset functions more effectively as a helper for B-cell activation. The type of immune response to an antigen is generally influenced by the cytokines produced by the cells responding to the antigen. Differences in the cytokines secreted by Th1 and Th2 cells are believed to reflect different biological functions of these two subsets. See, for example, Romagnani (2000) Ann. Allergy Asthma Immunol. 85:9-18.

Summary of Invention Paragraph:
[0013] Administration of an immunostimulatory polynucleotide with an antigen results in a Th1-type immune response to the administered antigen. Roman et al. (1997) Nature Med. 3:849-854. For example, mice injected intradermally with Escherichia coil (E. coli) .beta.-galactosidase (.beta.-Gal) in saline or in the adjuvant alum responded by producing specific IgG1 and IgE antibodies, and CD4.sup.+ cells that secreted IL-4 and IL-5, but not IFN-.gamma., demonstrating that the T cells were predominantly of the Th2 subset. However, mice injected intradermally (or with a tyne skin scratch applicator) with plasmid DNA (in saline) encoding .beta.-Gal and containing an ISS responded by producing IgG2a antibodies and CD4.sup.+ cells that secreted IFN-.gamma., but not IL-4 and IL-5, demonstrating that the T cells were predominantly of the Th1 subset. Moreover, specific IgE production by the plasmid DNA-injected mice was reduced 66-75%. Raz et al. (1996) Proc. Natl. Acad. Sci. USA 93:5141-5145. In general, the response to naked DNA immunization is characterized by production of IL-2, TNF.alpha. and IFN-.gamma. by antigen-stimulated CD4.sup.+ T cells, which is indicative of a Th1-type response. This is particularly important in treatment of allergy and asthma as shown by the decreased IgE production. The ability of immunostimulatory polynucleotides to stimulate a Th1-type immune response has been demonstrated with bacterial antigens, viral antigens and with allergens (see, for example, WO 98/55495).

L19: Entry 21 of 192 File: PGPB May 9, 2002

DOCUMENT-IDENTIFIER: US 20020055477 A1
TITLE: Immunomodulatory formulations and methods for use thereof

Pre-Grant Publication Year:
2002

Summary of Invention Paragraph:
[0002] The present invention relates to immunomodulatory compositions comprising an immunostimulatory oligonucleotide sequence (ISS). In particular, the invention relates to immunomodulatory compositions comprising an ISS bound to a nonbiodegradable microparticle. It also relates to the administration of the polynucleotide/microcarrier complex to modulate at least one immune response.

Summary of Invention Paragraph:
[0008] Administration of certain DNA sequences, generally known as immunostimulatory sequences or "ISS," induces an immune response with a Th1-type bias as indicated by secretion of Th1-associated cytokines. Administration of an immunostimulatory polynucleotide with an antigen results in a Th1-type immune response to the administered antigen. Roman et al. (1997) Nature Med. 3:849-854. For example, mice injected intradermally with Escherichia coli (E. coli) .beta.-galactosidase (.beta.-Gal) in saline or in the adjuvant alum responded by producing specific IgG1 and IgE antibodies, and CD4.sup.+ cells that secreted IL-4 and IL-5, but not IFN-.gamma., demonstrating that the T cells were predominantly of the Th2 subset. However, mice injected intradermally (or with a tyne skin scratch applicator) with plasmid DNA (in saline) encoding .beta.-Gal and containing an ISS responded by producing IgG2a antibodies and CD4.sup.+ cells that secreted IFN-.gamma., but not IL-4 and IL-5, demonstrating that the T cells were predominantly of the Th1 subset. Moreover, specific IgE production by the plasmid DNA-injected mice was reduced 66-75%. Raz et al. (1996) Proc. Natl. Acad. Sci. USA 93:5141-5145. In general, the response to naked DNA immunization is characterized by production of IL-2, TNF.alpha. and IFN-.gamma. by antigen-stimulated CD4.sup.+ T cells, which is indicative of a Th1-type response. This is particularly important in treatment of allergy and asthma as shown by the decreased IgE production. The ability of immunostimulatory polynucleotides to stimulate a Th1-type immune response has been demonstrated with bacterial antigens, viral antigens and with allergens (see, for example, WO 98/55495).

Summary of Invention Paragraph:
[0063] The invention provides new compositions for modulating immune response in individuals. The new compositions are immunomodulatory polynucleotide/microcarrier (IMP/MC) complexes which comprise an ISS-containing polynucleotide complexed to a nonbiodegradable microcarrier. IMP/MC complexes may be covalent complexes, in which the IMP portion of the complex is covalently bonded to the MC, either directly or via a linker (i.e., indirectly), or they may be non-covalent complexes.

CLAIMS:

12. A method of modulating an immune response in an individual comprising administering to an individual an immunomodulatory polynucleotide/microcarrier (IMP/MC) complex in an amount sufficient to modulate an immune response in said

individual, wherein said MC is a nonbiodegradable MC and wherein the ISS comprises the sequence 5'-C, G-3'.

24. A method of increasing interferon-gamma (IFN-.gamma.) in an individual, comprising: administering an effective amount of an immunomodulatory polynucleotide/microcarrier (IMP/MC) complex to said individual, wherein said MC is a nonbiodegradable MC, wherein the ISS comprises the sequence 5'-C, G-3' and wherein an effective amount is an amount sufficient to increase IFN-.gamma. in said individual.

35. A method of increasing interferon-alpha (IFN-.alpha.) in an individual, comprising: administering an effective amount of an immunomodulatory polynucleotide/microcarrier (IMP/MC) complex to said individual, wherein said MC is a nonbiodegradable MC, wherein the ISS comprises the sequence 5'-C, G-3' and wherein an effective amount is an amount sufficient to increase IFN-.alpha. in said individual.

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L19: Entry 32 of 192 File: USPT Jun 18, 2002

DOCUMENT-IDENTIFIER: US 6406705 B1
TITLE: Use of nucleic acids containing unmethylated CpG dinucleotide as an adjuvant

YEAR ISSUED (1):
2002

Brief Summary Text (4):
Bacterial DNA, but not vertebrate DNA, has direct immunostimulatory effects on peripheral blood mononuclear cells (PBMC) in vitro (Krieg et al., 1995). This lymphocyte activation is due to unmethylated CpG dinucleotides, which are present at the expected frequency in bacterial DNA (1/16), but are under-represented (CpG suppression, 1/50 to 1/60) and methylated in vertebrate DNA. Activation may also be triggered by addition of synthetic oligodeoxynucleotides (ODN) that contain an unmethylated CpG dinucleotide in a particular sequence context. It appears likely that the rapid immune activation in response to CpG DNA may have evolved as one component of the innate immune defense mechanisms that recognize structural patterns specific to microbial molecules.

Brief Summary Text (25):
FIG. 1 has two graphs illustrating humoral and cytotoxic T-lymphocyte (CTL) responses in adult BALB/c mice immunized with 1 .mu.g recombinant HBsAg protein alone, adsorbed onto alum (25 mg Al.sup.3+ /mg HBsAg), with 100 .mu.g of immunostimulatory CpG ODN 1826, or with both alum and CpG ODN. Left panel: Each point represents the group mean (n=10) for titers of anti-HBs (total IgG) as determined in triplicate by end-point dilution ELISA assay. End-point titers were defined as the highest plasma dilution that resulted in an absorbance value (OD 450) two times greater than that of control non-immune plasma with a cut-off value of 0.05. Rightpanel: Each point represents the mean % specific lysis at the indicated effector: target (E:T) cell ratio in a chromium release assay with HBsAg-expressing cells as targets.

Brief Summary Text (33):
FIG. 9 has two graphs illustrating humoral and cytotoxic T-lymphocyte (CTL) responses in BALB/c mice immunized at 7 days of age with a DNA vaccine (1 .mu.g pCMV-S), or with 1 .mu.g recombinant HBsAg protein alone, adsorbed onto alum (25 mg Al.sup.3+ /mg HBsAg), with 100 .mu.g of immunostimulatory CpG ODN 1826, or with both alum and CpG ODN. Upper panel: Each point represents the group mean of animals that seroconverted (see FIG. 8 for numbers of animals) for titers of anti-HBs (total IgG) as determined in triplicate by end-point dilution ELISA assay. End-point titers were defmed as the highest plasma dilution that resulted in an absorbance value (OD 450) two times greater than that of control non-immune plasma with a cut-off value of 0.05. Lower panel: Each point represents the mean % specific lysis at the indicated effector: target (E:T) cell ratio in a chromium release assay with HBsAg-expressing cells as targets.

Brief Summary Text (34):
FIG. 10 is a bar graph illustrating humoral responses in neonatal BALB/c mice at 8 weeks after immunization (at 7 days of age) with 1 .mu.g recombinant HBsAg protein with alum (25 mg Al.sup.3+ /mg HBsAg), with 10 .mu.g of CpG ODN 1826, or with both alum and CpG ODN. Each point represents the group mean (see FIG. 8 for numbers of

animals) for anti-HBs titers (IgG1 and IgG2a isotypes) as determined by end-point dilution ELISA assay. IgG1 antibodies indicate a Th2-biased response whereas IgG2a antibodies are indicative of a Th1-type response.

Detailed Description Text (3):
It has been discovered according to the invention that the combination of immunostimulatory CpG oligonucleotides and alum, MPL and other adjuvants results in a synergistic immune response. Compared with the recombinant hepatitis B surface antigen (HBsAg) protein vaccine alone, addition of alum increases the level of antibodies in mice against HBsAg (anti-HBs) about 7-fold whereas addition of CpG ODN increases them 32-fold. When CpG ODN and alum are used together, a 500-1000 times higher level of anti-HBs was observed, indicating a strong synergistic response. Additionally, it was found according to the invention that immunization with HBsAg and alum resulted in a strong Th2-type response with almost all IgG being of the IgG1 isotype. CpG ODN induced a high proportion of IgG2a, indicative of a Th1-type response, even in the presence of alum. Furthermore, it was discovered according to the invention that in very young mice (7 day old), immune responses were induced by HBsAg with alum and CpG ODN but not with alum or CpG ODN alone. The antibodies produced with CpG ODN were predominantly of the IgG2a isotype, indicating a strong Th1-type response. This is remarkable considering the strong Th2 bias of the neonatal immune system and the known difficulty in inducing Th1 responses at such a young age. Th1 responses are preferable in some instances since they are associated with IgG2a antibodies that have better neutralization and opsonization capabilities than Th2-type antibodies. As well, Th1 responses are associated with cytotoxic T lymphocytes (CTL) that can attack and kill virus-infected cells. Indeed, CpG ODN, alone or in combination with alum induced good CTL activity in both adult and neonatal mice. These studies demonstrate that the addition of CpG ODN to protein or DNA vaccines in combination with other adjuvants is a valid new adjuvant approach to improve efficacy.

Detailed Description Text (99):
The CpG ODN of the invention stimulate cytokine production (e.g., IL-6, IL-1 2, IFN-.gamma., TNF-.alpha. and GM-CSF) and B-cell proliferation in PBMC's taken from a subject such as a human. Specific, but nonlimiting examples of such sequences include those presented in Table 1 below:

Detailed Description Text (100):
Preferred CpG ODN can effect at least about 500 pg/ml of TNF-.alpha., 15 pg/ml IFN-.gamma., 70 pg/ml of GM-CSF 275 pg/ml of IL-6, 200 pg/ml IL-12, depending on the therapeutic indication. These cytokines can be measured by assays well known in the art. The oligonucleotides listed above or other preferred CpG ODN can effect at least about 10%, more preferably at least about 15% and most preferably at least about 20% YAC-1 cell specific lysis or at least about 30%, more preferably at least about 35%, and most preferably at least about 40% 2C11 cell specific lysis, in assays well known in the art.

Detailed Description Text (151):
Similar synergistic results for antibody responses were obtained when immunization against HBsAg was carried out in neonatal and very young mice, in which the immune system is immature. In mice immunized at 3 days of age, where the immune system is even less mature than a newborn human, 10% and 0% of mice seroconverted with alum and CpG ODN alone respectively, but 75% serocoinverted when CpG ODN and alum were used together. In 7 day old mice, which have an immune system similar in maturity to that of a newborn human, seroconversion for alum, CpG or the combination was 11%, 22% and 1 00% respectively (FIG. 8). Furthermore, in these 7 day old mice, antibody titers were up to 80-fold higher with the combined adjuvants than with either adjuvant alone (FIG. 9).

Detailed Description Text (157):
Thus, CpG ODN is superior to alum for both humoral and cell-mediated responses,

when each is used alone as adjuvant with the HBsAg subunit vaccine in mice. When used together, there is a synergy of action such that antibody and CTL activity are stronger than when either adjuvant is used alone. These results indicate that CpG ODN could be used to replace alum in vaccine formulations, which could be desirable to avoid associated side-effects due to local irritation in the muscle, or for certain live-attenuated or multivalent vaccines where it is not possible to use alum because chemical interactions interfere with the efficacy of the vaccine. This should not occur with CpG ODN. Of even greater interest is the strong synergistic response when CpG ODN and alum are used together as adjuvants. This could allow better immune responses with lower or fewer doses of antigen. There is a fairly flat dose response to CpG ODN whether or not alum is present, indicating that a wide range of CpG ODN could be useful to adjuvant vaccines in humans.

Detailed Description Text (162):
Similarly, CpG ODN and MPL alone gave equally high antibody titers, but when used together the titers were about 4-times higher than with either adjuvant alone (FIG. 7). While the synergistic response with CpG and MPL was not as marked with respect to overall antibody titers, it was very pronounced with respect to the Th1-bias of these antibodies (see below).

Detailed Description Text (164):
Dominance and Synergy of CpG ODN with Alum for Induction of a Th1-type immune response including CTL

Detailed Description Text (165):
Immunization with either HBsAg alone or with alum induces a predominantly Th2-type humoral response with virtually no IgG2a antibodies, which are induced in response to Th1 -type cytokines such as IL-12 and IFN-.gamma.. Rather, almost all (>99%) antibodies were of the IgG1 isotype IgG2a:IgG1=0.01. CpG ODN induces significantly more IgG2a antibodies, such that they made up at least 50% of the total IgG (IgG (IgG2a:IgG1=1.4). The combination of alum and CpG ODN induce an equally strong Th1 response as CpG ODN alone (IgG2a:IgG1=1.0), despite the extremely strong Th2-bias of alum (FIG. 5). Similarly CTL responses with CpG ODN plus alum were as strong as those with CpG ODN alone, despite the fact that the Th2-bias of alum resulted in a complete loss of CTL when alum was used alone (FIG. 1).

Detailed Description Text (166):
The strong Th1 bias with CpG is even more evident in neonatal and young mice, which are known to naturally have a strong Th2-bias to their immune system. In this case, neither alum nor CpG ODN on their own induced detectable IgG2a, indicating a very poor or absent Th1 response. Remarkably, when used together, CpG ODN and alum induced high levels of Ig G2a antibodies, which were now the predominant form of IgG (FIG. 10). Similarly, neither CpG ODN or alum induced significant levels of CTL in young mice, yet when used together there was a strong CTL response, that was even stronger than obtained with a DNA vaccine (FIG. 9).

Detailed Description Text (167):
The strength of the Th1 influence of CpG ODN is seen not only by its ability to dominate over the Th2 effect of alum when they are co-administered, but also to induce Th1 responses in animals previously primed for a Th2 response with alum. Immunization with HBsAg using alum as an adjuvant completely abrogates the CTL response owing to the strong Th2 bias of alum (FIGS. 1 and 4). However, in mice using alum at prime and CpG at boost, good CTL were induced, indicating the possibility of CpG to overcome a previously established Th2 response (FIG. 4).

Detailed Description Text (168):
Aluminum hydroxide (alum) is currently the only adjuvant approved for human use. An important disadvantage of alum is that it induces a Th2- rather than a Th1-type immune response, and this may interfere with induction of CTL. Indeed, in mice immunized with recombinant HBsAg, the addition of alum selectively blocked

activation of CD8.sup.+ CTL (Schirmbeck et al., 1994). Although not essential for protective immunity against HBV, CTL may nevertheless play an important role. For example, a lack of HBV-specific CTL is thought to contribute to the chronic carrier state. In contrast, one of the primary advantages of CpG DNA over alum as an adjuvant is the Th1-bias of the responses and thus the possibility to induce CTL. A striking finding from the present study is that CpG can completely counteract the Th2-bias of alum when the two adjuvants are delivered together, and in the case of immunization in early life, the combination can even give a more Th1 response than CpG ODN alone. This could allow one to capitalize on the strong synergistic action of the two adjuvants on the humoral response while still allowing CTL in adults, and to induce a stronger Th1 response in infants.

Detailed Description Text (169):
The use of alum has been linked to Th2-type diseases. The much higher prevalence of asthma (another Th2-type disease) in more highly developed nations may be linked to the high hygiene level and rapid treatment of childhood infections (Cookson and Moffatt, 1997). Early exposure to bacterial DNA (and immunostimulatory CpG motifs) pushes the immune system away from Th2- and towards a Th1-type response and this may account for the lower incidence of asthma in less developed countries, where there is a much higher frequency of upper respiratory infections during childhood. Addition of CpG ODN as adjuvant to all pediatric vaccines could re-establish a Th1-type response thereby reducing the incidence of asthma.

Detailed Description Text (171):
Synergy of CpG ODN with Other Adjuvants for Induction of a Th1-type Immune Responses

Detailed Description Text (172):
The synergistic effect of CpG ODN on Th1 responses was also seen using other adjuvants. IFA on its own induces a very strong Th2-type response with virtually no IgG2a antibodies (IgG2a:IgG1=0.002) and CpG ODN on its own induces a moderate Th1 response (IgG2a:IgG1=1.4), but together the response was very strongly Th1 (IgG2a:IgG1=24.0). It is notable that this is even more Th1 than the response induced by CFA (ratio=0.5) (FIG. 6).

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L19: Entry 36 of 192 File: USPT Dec 25, 2001

DOCUMENT-IDENTIFIER: US 6333314 B1
** See image for Certificate of Correction **
TITLE: Liposomes containing oligonucleotides

YEAR ISSUED (1):
2001

Detailed Description Text (38):
Radiation survival dose responses of SQ-20B cells exposed to S- and As-ODNs (100 pmol/.mu.l, 12 hr) were evaluated. (S- and As-ODNs used in this study do not contain the G-quartet or CpG motifs previously shown to be responsible for non-antisense-specific effects such as enhanced affinity for protein or interference with the immune response.) The plating efficiencies indicated that the As-ODNs treatment had no effect on cell viability as compared to S-ODNs-treated cells (Table 1). These data are also in agreement with the S- and As-ODNs effects on the viability of logarithmically growing cells discussed earlier. Radiation survival dose responses of the control (without oligo) and S-ODNs-treated cells were almost identical. Most important, As-ODNs treatment resulted in decreases of the shoulder and the slope of the survival curve. The radiobiological parameters were obtained by fitting the data (surviving number of colonies) to the single-hit multitarget (D.sub.0, D.sub.q, n) and linear-quadratic (.alpha., .beta.,) models of radiation survival response. In addition, the value of a model-free parameter, mean inactivation dose (D) was calculated (14) (Table 1). Based on a ratio of the mean inactivation dose, the dose modifying factor (DMF) of As-ODNs treatment was .sup.18 1.4. Significant decreases observed in the values of radiobiological parameters, D, D.sub.q, and D.sub.0 of SQ-20B cells following treatment with the raf As-ODNs indicate a good correlation between the DNA sequence-specific inhibition of Raf-1 protein kinase and the radiosensitization of these relatively radioresistant tumor cells.

L19: Entry 43 of 192 File: USPT Apr 10, 2001

DOCUMENT-IDENTIFIER: US 6214806 B1
** See image for Certificate of Correction **
TITLE: Use of nucleic acids containing unmethylated CPC dinucleotide in the treatment of LPS-associated disorders

YEAR ISSUED (1):
2001

Drawing Description Text (9):
FIG. 8 is a graph plotting lung lavage cell count over time. The graph shows that when the mice are initially injected intraperitoneally (i.p.) with Schistosoma mansoni eggs "egg," which induces a Th2 immune response, and subsequently inhale Schistosoma mansoni egg antigen "SEA" (open circle), many inflammatory cells are present in the lungs. However, when the mice are initially given CpG ODN along with egg, the inflammatory cells in the lung are not as increased by subsequent inhalation of SEA (open triangles).

Drawing Description Text (13):
FIG. 12 is a graph plotting interleukin 4 (IL-4) production pg/ml) in mice over time in response to injection of egg, then SEA (open diamond); egg and CpG ODN, then SEA (open circle); or saline, then saline (open square). The graph shows that the resultant inflammatory response correlates with the levels of the Th2 cytokine IL-4 in the lung.

Drawing Description Text (14):
FIG. 13 is a bar graph plotting interleukin 12 (IL-12) production (pg/ml) in mice over time in response to injection of saline; egg, then SEA; or CpG ODN and egg, then SEA. The graph shows that administration of an oligonucleotide containing an unmethylated CpG motif can actually redirect the cytokine response of the lung to production of IL-12, indicating a Th1 type of immune response.

Drawing Description Text (15):
FIG. 14 is a bar graph plotting interferon gamma (IFN-.gamma. production (pg/ml) in mice over time in response to injection of saline; egg, then saline; or CpG ODN and egg, then SEA. The graph shows that administration of an oligonucleotide containing an unmethylated CpG motif can also redirect the cytokine response of the lung to production of IFN-.gamma., indicating a Th1 type of immune response.

Detailed Description Text (4):
The binding of DNA to cells has been shown to be similar to a ligand receptor interaction: binding is saturable, competitive, and leads to DNA endocytosis and degradation into oligonucleotides (Bennet, R. M., et al., J. Clin. Invest. 76:2182, 1985). Like DNA, oligodeoxyribonucleotides are able to enter cells in a process which is sequence, temperature, and energy independent (Jaroszewski and Cohen, Ad. Drug Del. Rev. 6:235, 1991). An "oligodeoxyribonycleotide" as used herein is a deoxyribonucleic acid sequence from about 3-50 bases in length. Lymphocyte oligodeoxyribonucleotide uptake has been shown to be regulated by cell activation (Krieg, A. M., et al., Antisense Research and Development 1:161, 1991). The present invention is based on the finding that certain oligonucleotides (ODN) containing at least one unmethylated cytosine-guanine (CpG) dinucleotide activate the immune

response.

Detailed Description Text (16):
The "stimulation index" is a measure of a CpG ODN to effect an immune response which can be tested in various immune cell assays. The stimulation of the immune response can be assayed by measuring various immune parameters, e.g., measuring the antibody-forming capacity, number of lymphocyte subpopulations, mixed leukocyte response assay, lymphocyte proliferation assay. The stimulation of the immune response can also be measured in an assay to determine resistance to infection or tumor growth. Methods for measuring a stimulation index are well known to one of skill in the art. For example, one assay is the incorporation of .sup.3 H uridine in a murine B cell culture, which has been contacted with a 20 .mu.M of oligonucleotide for 20 h at 37.degree. C. and has been pulsed with 1 .mu.Ci of .sup.3 H uridine; and harvested and counted 4 h later. The induction of secretion of a particular cytokine can also be used to assess the stimulation index. Without meaning to be bound by theory, for use in vivo, for example to treat a subject having or at risk of having an acute decrement in air flow in response to endotoxin, it is important that the CpG ODN be capable of effectively inducing cytokine secretion by monocytic cells and/or Natural Killer (NK) cell lytic activity. In one method, the stimulation index of the CpG ODN with regard to B-cell proliferation is at least about 5, preferably at least about 10, more preferably at least about 15 and most preferably at least about 20, while recognizing that there are differences in the stimulation index among individuals.

Detailed Description Text (17):
The CpG ODN of the invention stimulate cytokine production (e.g., IL-6, IL-12, IFN-.gamma., TNF-.alpha. and GM-CSF). Exemplary sequences include:

Detailed Description Text (23):
Preferred CpG ODN can effect at least about 500 pg/ml of TNF-.alpha., 15 pg/ml IFN-.gamma., 70 pg/ml of GM-CSF 275 pg/ml of IL-6, 200 pg/ml IL-12, depending on the therapeutic indication. These cytokines can be measured by assays well known in the art. The ODNs listed above or other preferred CpG ODN can effect at least about 10%, more preferably at least about 15% and most preferably at least about 20% YAC-1 cell specific lysis or at least about 30%, more preferably at least about 35%, and most preferably at least about 40% 2C11 cell specific lysis, in assays well known in the art (see Example 4).

Detailed Description Text (59):
CpG ODN Reduces the Pulmonary Response to Inhaled LPS and Stimulates the Immune Response

Detailed Description Text (61):
Compared to non-CpG ODN, CpG ODN resulted in an increase in the concentration of MIP-2, IL-10, and IL-12 in the serum of mice following LPS inhalation (FIG. 2). These differences were most pronounced 30 min and 4 hours after intravenous administration but were still present 12 hours after exposure to CpG containing oligonucleotides. No differences were observed for the serum concentration of TNF-.alpha., IL-6, and IFN-.gamma. at any of the time points in mice pre-treated with either oligonucleotide and then exposed to LPS (data not shown for IL-6).

Detailed Description Text (76):
Human peripheral blood mononuclear leukocytes (PBMC) were obtained as previously described (e.g., Ballas, Z. K. et al., J. Allergy Clin. Immunol. 85:453, 1990). Human or murine cells were cultured at 5.times.10.sup.6 /well, at 37.degree. C. in a 5% CO.sub.2 humidified atmosphere in 24-well plates with medium alone or with CpG or non-CpG ODN at the indicated concentrations, or with E. coli or calf thymus (50 .mu.g/ml) at 37.degree. C. for 24 hr. All cultures were harvested at 18 hr. and the cells were used as effectors in a standard 4 hr. .sup.51 Cr-release assay against K562 (human) or YAC-1 (mouse) target cells as previously described. For

calculation of lytic units (LU), 1 LU was defined as the number of cells needed to effect 30% specific lysis. Where indicated, neutralizing antibodies against IFN-.gamma. (Lee Biomolecular, San Diego, Calif.) or IL-12 (Pharmingen) or their isotype controls were added at the initiation of cultures to a concentration of 10 .mu.g/ml. For anti-IL-12 addition, 10 .mu.g of each of the 4 MAB (or isotype controls) were added simultaneously. Recombinant human IL-2 was used at a concentration of 100 U/ml.

Detailed Description Text (82):
Bacterial DNA cultured for 18 hrs. at 37.degree. C. and then assayed for killing of K562 (human) or Yac-1 (mouse) target cells induced NK lytic activity in both mouse spleen cells depleted of B cells, and human PBMC, but vertebrate DNA did not (Table 3). To determine whether the stimulatory activity of bacterial DNA may be a consequence of its increased level of unmethylated CpG dinucleotides, the activating properties of more than 50 synthetic ODN containing unmethylated, methylated, or no CpG dinucleotides was tested. The results, summarized in Table 3, demonstrate that synthetic ODN can stimulate significant NK activity, as long as they contain at least one unmethylated CpG dinucleotide (Ballas, Z., et al., J. Immunol 157:1840-1845, 1996). No difference was observed in the stimulatory effects of ODN in which the CpG was within a palindrome (such as ODN 1585, which contains the palindrome AACGTT) from those ODN without palindromes (such as 1613 or 1619), with the caveat that optimal stimulation was generally seen with ODN in which the CpG was flanked by two 5' purines or a 5' GpT dinucleotide and two 3' pyrimidines. Kinetic experiments demonstrated that NK activity peaked around 18 hrs. after addition of the ODN. The data indicates that the murine NK response is dependent on the prior activation of monocytes by CpG DNA, leading to the production of IL-12, TNF-.alpha., and IFN.

Detailed Description Text (83):
Immune activation by CpG motifs may depend on bases flanking the CpG, and the number and spacing of the CpGs present within an ODN. Although a single CpG in an ideal base context can be a very strong and useful immune activator, superior effects can be seen with ODN containing several CpGs with the appropriate spacing and flanking bases. For activation of murine B cells, the optimal CpG motif is TGACGTT.

Detailed Description Text (94):
The ability of a CpG ODN to induce IL-12 secretion is a good measure of its adjuvant potential, especially in terms of its ability to induce a Th1 immune response, which is highly dependent on IL-12. Therefore, the ability of a panel of phosphorothioate ODN to induce IL-12 secretion from human PBMC in vitro (Table 7) was examined. These experiments showed that in some human PBMC, most CpG ODN could induce IL-12 secretion (e.g., expt. 1). However, other donors responded to just a few CpG ODN (e.g., expt. 2). ODN 2006 was a consistent inducer of IL12 secretion from most subjects (Table 7).

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L19: Entry 44 of 192 File: USPT Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207646 B1
TITLE: Immunostimulatory nucleic acid molecules

YEAR ISSUED (1):
2001

Detailed Description Text (17):
The stimulation index of a particular immunostimulatory CpG DNA can be tested in various immune cell assays. Preferably, the stimulation index of the immunostimulatory CpG DNA with regard to B-cell proliferation is at least about 5, preferably at least about 10, more preferably at least about 15 and most preferably at least about 20 as determined by incorporation of .sup.3 H uridine in a murine B cell culture, which has been contacted with a 20 .mu.M of ODN for 20 h at 37.degree. C. and has been pulsed with 1 .mu.Ci of .sup.3 H uridine; and harvested and counted 4 h later as described in detail in Example 1. For use in vivo, for example to treat an immune system deficiency by stimulating a cell-mediated (local) immune response in a subject, it is important that the immunostimulatory CpG DNA be capable of effectively inducing cytokine secretion by monocytic cells and/or Natural Killer (NK) cell lytic activity.

Detailed Description Text (39):
Certain B cell lines such as WEHI-231 are induced to undergo growth arrest and/or apoptosis in response to crosslinking of their antigen receptor by anti-IgM (Jakway, J. P. et al., "Growth regulation of the B lymphoma cell line WEHI-231 by anti-immunoglobulin, lipopolysaccharide and other bacterial products" J. Immunol. 137: 2225 (1986); Tsubata, T., J. Wu and T. Honjo: B-cell apoptosis induced by antigen receptor crosslinking is blocked by a T-cell signal through CD40." Nature 364: 645 (1993)). WEHI-231 cells are rescued from this growth arrest by certain stimuli such as LPS and by the CD40 ligand. ODN containing the CpG motif were also found to protect WEHI-231 from anti-IgM induced growth arrest, indicating that accessory cell populations are not required for the effect. Subsequent work indicates that CpG ODN induce Bcl-x and myc expression, which may account for the protection from apoptosis. Also, CpG nucleic acids have been found to block apoptosis in human cells. This inhibition of apoptosis is important, since it should enhance and prolong immune activation by CpG DNA.

Detailed Description Text (55):
In vivo or in whole spleen cells, no significant increase in the protein levels of the following interleukins: IL-2, IL-3, IL-4, IL-5, or IL-10 was detected within the first six hours (Klinman, D. M. et al., (1996) Proc. Natl. Acad. Sci. USA 93:2879-2883). However, the level of TNF-.alpha. is increased within 30 minutes and the level of IL-6 increased strikingly within 2 hours in the serum of mice injected with CpG ODN. Increased expression of IL-12 and interferon gamma (IFN-.gamma.) mRNA by spleen cells was also detected within the first two hours.

Detailed Description Text (57):
The same panels of ODN used for studying mouse cytokine expression were used to determine whether human cells also are induced by CpG motifs to express cytokine (or proliferate), and to identify the CpG motif(s) responsible. Oligonucleotide 1619 (GTCGTT) was the best inducer of TNF-.alpha. and IFN-.gamma. secretion, and

was closely followed by a nearly identical motif in oligonucleotide 1634 (GTCGCT) (Table 5). The motifs in oligodeoxynucleotides 1637 and 1614 (GCCGGT and GACGGT) led to strong IL-6 secretion with relatively little induction of other cytokines. Thus, it appears that human lymphocytes, like murine lymphocytes, secrete cytokines differentially in response to CpG dinucleotides, depending on the surrounding bases. Moreover, the motifs that stimulate murine cells best differ from those that are most effective with human cells. Certain CpG oligodeoxynucleotides are poor at activating human cells (oligodeoxynucleotides 1707, 1708, which contain the palindrome forming sequences GACGTC and CACGTG respectively).

Detailed Description Text (68):
Phosphorothioate modified ODN (S-ODN) are far more nuclease resistant than phosphodiester modified ODN (O-ODN). Thus, the increased immune stimulation caused by S-ODN and S-O-ODN (i.e. chimeric phosphorothioate ODN in which the central linkages are phosphodiester, but the two 5' and five 3' linkages are phosphorothioate modified) compared to O-ODN may result from the nuclease resistance of the former. To determine the role of ODN nuclease resistance in immune stimulation by CpG ODN, the stimulatory effects of chimeric ODN in which the 5' and 3' ends were rendered nuclease resistant with either methylphosphonate (MP-), methylphosphorothioate (MPS-), phosphorothioate (S-), or phosphorodithioate (S.sub.2 -) internucleotide linkages were tested (Example 10). These studies showed that despite their nuclease resistance, MP-O-ODN were actually less immune stimulatory than O-ODN. However, combining the MP and S modifications by replacing both nonbridging O molecules with 5' and 3' MPS internucleotide linkages restored immune stimulation to a slightly higher level than that triggered by O-ODN.

Detailed Description Text (69):
S-O-ODN were far more stimulatory than O-ODN, and were even more stimulatory than S-ODN, at least at concentrations above 3.3 .mu.M. At concentrations below 3 .mu.M, the S-ODN with the 3M sequence was more potent than the corresponding S-O-ODN, while the S-ODN with the 3D sequence was less potent than the corresponding S-O-ODN (Example 10). In comparing the stimulatory CpG motifs of these two sequences, it was noted that the 3D sequence is a perfect match for the stimulatory motif in that the CpG is flanked by two 5' purines and two 3' pyrimidines. However, the bases immediately flanking the CpG in ODN 3D are not optimal; it has a 5' pyrimidine and a 3' purine. Based on further testing, it was found that the sequence requirement for immune stimulation is more stringent for S-ODN than for S-O- or O-ODN. S-ODN with poor matches to the optimal CpG motif cause little or no lymphocyte activation (e.g. Sequence 3D). However, S-ODN with good matches to the motif, most critically at the positions immediately flanking the CpG, are more potent than the corresponding S-O-ODN (e.g. Sequence 3M, Sequences 4 and 6), even though at higher concentrations (greater than 3 .mu.M) the peak effect from the S-O-ODN is greater (Example 10).

Detailed Description Text (71):
The increased B cell stimulation seen with CpG ODN bearing S or S.sub.2 substitutions could result from any or all of the following effects: nuclease resistance, increased cellular uptake, increased protein binding, and altered intracellular localization. However, nuclease resistance can not be the only explanation, since the MP-O-ODN were actually less stimulatory than the O-ODN with CpG motifs. Prior studies have shown that ODN uptake by lymphocytes is markedly affected by the backbone chemistry (Zhao et al., (1993) Comparison of cellular binding and uptake of antisense phosphodiester, phosphorothioate, and mixed phosphorothioate and methylphosphonate oligonucleotides. (Antisense Research and Development 3, 53-66; Zhao et al., (1994) Stage specific oligonucleotide uptake in murine bone marrow B cell precursors. Blood 84, 3660-3666.) The highest cell membrane binding and uptake was seen with S-ODN, followed by S-O-ODN, O-ODN, and MP-ODN. This differential uptake correlates well with the degree of immune stimulation.

Detailed Description Text (75):
Bacterial DNA cultured for 18 hrs. at 37.degree. C. and then assayed for killing of K562 (human) or Yac-1 (mouse) target cells induced NK lytic activity in both mouse spleen cells depleted of B cells and human PBMC, but vertebrate DNA did not (Table 9). To determine whether the stimulatory activity of bacterial DNA may be a consequence of its increased level of unmethylated CpG dinucleotides, the activating properties of more than 50 synthetic ODN containing unmethylated, methylated, or no CpG dinucleotides was tested. The results, summarized in Table 9, demonstrate that synthetic ODN can stimulate significant NK activity, as long as they contain at least one unmethylated CpG dinucleotide. No difference was observed in the stimulatory effects of ODN in which the CpG was within a palindrome (such as ODN 1585, which contains the palindrome AACGTT) from those ODN without palindromes (such as 1613 or 1619), with the caveat that optimal stimulation was generally seen with ODN in which the CpG was flanked by two 5' purines or a 5' GpT dinucleotide and two 3' pyrimidines. Kinetic experiments demonstrated that NK activity peaked around 18 hrs. after addition of the ODN. The data indicates that the murine NK response is dependent on the prior activation of monocytes by CpG DNA, leading to the production of IL-12, TNF-.alpha., and IFN-.alpha./.beta. (Example 11).

Detailed Description Text (167):
Human peripheral mononuclear blood leukocytes (PBMC) were obtained as previously described (Ballas, Z. K. et al., (1990) J. Allergy Clin. Immunol. 85:453; Ballas, Z. K. and W. Rasmussen (1990) J. Immunol. 145:1039; Ballas, Z. K. and W. Rasmussen (1993) J. Immunol. 150;17). Human or murine cells were cultured at 5.times.10.sup.6 /well, at 37.degree. C. in a 5% CO.sub.2 humidified atmosphere in 24-well plates (Ballas, Z. K. et al., (1990) J. Allergy Clin. Immunol. 85:453; Ballas, Z. K. and W. Rasmussen (1990) J. Immunol 145:1039; and Ballas, Z. K. and W. Rasmussen (1993) J. Immunol, 150:17), with medium alone or with CpG or non-CpG ODN at the indicated concentrations, or with E. coli or calf thymus (50 .mu.g/ml) at 37.degree. C. for 24 hr. All cultures were harvested at 18 hr. and the cells were used as effectors in a standard 4 hr. .sup.51 Cr-release assay against K562 (human) or YAC-1 (mouse) target cells as previously described. For calculation of lytic units (LU), 1 LU was defined as the number of cells needed to effect 30% specific lysis. Where indicated, neutralizing antibodies against IFN-.beta. (Lee Biomolecular, San Diego, Calif.) or IL-12 (C15.1, C15.6, C17.8, and C17.15; provided by Dr. Giorgio Trinchieri, The Wistar Institute, Philadelphia, Pa.) or their isotype controls were added at the initiation of cultures to a concentration of 10 .mu.g/ml. For anti-IL-12 addition, 10 .mu.g of each of the 4 MAB (or isotype controls) were added simultaneously. Recombinant human IL-2 was used at a concentration of 100 U/ml.

Detailed Description Paragraph Table (5):
TABLE 5 Induction of human PBMC cytokine secrtetion by CpG oligos ODN Sequence (5'-3') IL-6.sup.1 TNF-.alpha..sup.1 IFN-.gamma..sup.1 GM-CSF IL-12 512
TCCATGTCGGTCCTGATGCT 500 140 15.6 70 250 SEQ ID NO:37 1637 ......C..... 550
16 7.8 15.6 35 SEQ ID NO:38 1615 ......G..... 600 145 7.8 45 250 SEQ ID
NO:39 1614 ......A..... 550 31 0 50 250 SEQ ID NO:40
1636 .....A..... 325 250 35 40 0 SEQ ID NO:41 1634 .....C.....
300 400 40 85 200 SEQ ID NO:42 1619 .....T..... 275 450 200 80 >500 SEQ ID
NO:43 1618 ......A..T..... 300 60 15.6 15.6 62 SEQ ID NO:44
1639 .....AA..T..... 625 220 15.6 40 60 SEQ ID NO:45 1707 ......A..TC.....
300 70 17 0 0 SEQ ID NO:46 1708 .....CA..TG..... 270 10 17 0 0 SEQ ID NO:47
dots indicate identity; CpG dinucleotides are underlined .sup.1 measured by ELISA using Quantikine kits from R&D Systems (pg/ml) Cells were cultured in 10% autologous serum with the indicated oligodeoxynucleotides (12 .mu.g/ml) for 4 hr in the case of TN-.alpha.or 24 hr for the other cytokines before supernatant harvest and assay. Data are presented as the level of cytokine above that in wells with no added oligodeoxynucleotide.

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L19: Entry 44 of 192 File: USPT Mar 27, 2001

DOCUMENT-IDENTIFIER: US 6207646 B1
TITLE: Immunostimulatory nucleic acid molecules

YEAR ISSUED (1):
2001

Detailed Description Text (17):
The stimulation index of a particular immunostimulatory CpG DNA can be tested in various immune cell assays. Preferably, the stimulation index of the immunostimulatory CpG DNA with regard to B-cell proliferation is at least about 5, preferably at least about 10, more preferably at least about 15 and most preferably at least about 20 as determined by incorporation of .sup.3 H uridine in a murine B cell culture, which has been contacted with a 20 .mu.M of ODN for 20 h at 37.degree. C. and has been pulsed with 1 .mu.Ci of .sup.3 H uridine; and harvested and counted 4 h later as described in detail in Example 1. For use in vivo, for example to treat an immune system deficiency by stimulating a cell-mediated (local) immune response in a subject, it is important that the immunostimulatory CpG DNA be capable of effectively inducing cytokine secretion by monocytic cells and/or Natural Killer (NK) cell lytic activity.

Detailed Description Text (39):
Certain B cell lines such as WEHI-231 are induced to undergo growth arrest and/or apoptosis in response to crosslinking of their antigen receptor by anti-IgM (Jakway, J. P. et al., "Growth regulation of the B lymphoma cell line WEHI-231 by anti-immunoglobulin, lipopolysaccharide and other bacterial products" J. Immunol. 137: 2225 (1986); Tsubata, T., J. Wu and T. Honjo: B-cell apoptosis induced by antigen receptor crosslinking is blocked by a T-cell signal through CD40." Nature 364: 645 (1993)). WEHI-231 cells are rescued from this growth arrest by certain stimuli such as LPS and by the CD40 ligand. ODN containing the CpG motif were also found to protect WEHI-231 from anti-IgM induced growth arrest, indicating that accessory cell populations are not required for the effect. Subsequent work indicates that CpG ODN induce Bcl-x and myc expression, which may account for the protection from apoptosis. Also, CpG nucleic acids have been found to block apoptosis in human cells. This inhibition of apoptosis is important, since it should enhance and prolong immune activation by CpG DNA.

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was closely followed by a nearly identical motif in oligonucleotide 1634 (GTCGCT) (Table 5). The motifs in oligodeoxynucleotides 1637 and 1614 (GCCGGT and GACGGT) led to strong IL-6 secretion with relatively little induction of other cytokines. Thus, it appears that human lymphocytes, like murine lymphocytes, secrete cytokines differentially in response to CpG dinucleotides, depending on the surrounding bases. Moreover, the motifs that stimulate murine cells best differ from those that are most effective with human cells. Certain CpG oligodeoxynucleotides are poor at activating human cells (oligodeoxynucleotides 1707, 1708, which contain the palindrome forming sequences GACGTC and CACGTG respectively).

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Detailed Description Text (167):
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1636 .....A..... 325 250 35 40 0 SEQ ID NO:41 1634 .....C..... 300 400 40 85 200 SEQ ID NO:42 1619 .....T..... 275 450 200 80 >500 SEQ ID NO:43 1618 ......A..T..... 300 60 15.6 15.6 62 SEQ ID NO:44
1639 .....AA..T..... 625 220 15.6 40 60 SEQ ID NO:45 1707 ......A..TC..... 300 70 17 0 0 SEQ ID NO:46 1708 .....CA..TG..... 270 10 17 0 0 SEQ ID NO:47
dots indicate identity; CpG dinucleotides are underlined .sup.1 measured by ELISA using Quantikine kits from R&D Systems (pg/ml) Cells were cultured in 10% autologous serum with the indicated oligodeoxynucleotides (12 .mu.g/ml) for 4 hr in the case of TN-.alpha.or 24 hr for the other cytokines before supernatant harvest and assay. Data are presented as the level of cytokine above that in wells with no added oligodeoxynucleotide.

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L19: Entry 64 of 192 File: DWPI Jul 8, 2003

DERWENT-ACC-NO: 2001-536419
DERWENT-WEEK: 200347

TITLE: Pharmaceutical composition useful for inducing immune response comprises antigen, immunogenic oligodeoxynucleotide containing cytosine-guanine dinucleotide motifs and polycationic polymer

Basic Abstract Text (6):
Mice were injected into each hind footpad with a total volume of 100 mu l, 50 mu l per footpad, containing CpG-ODN. Animals were sacrificed 4 days after injection and popliteal lymph nodes were harvested. Lymph nodes were harvested and passed through 70 mu m cell strainer and washed with DMEM containing 5% fetal calf serum. Cells were adjusted to 107 cells/ml in DMEM/5%FCS. IFN- gamma -ELISPOT assay were carried out in duplicates. While injection of the peptides with poly-L-arginine or CpG-ODN alone leads to no or only low numbers of peptide-specific IFN- gamma -producing cells, the injection of peptides with the combination of poly-L-arginine and CpG-ODN induced or strongly enhanced the peptide-specific response. Using the non-immunogenic Non-CpG-ODN instead of CpG-ODN, the application of poly-L-arginine had no increasing effect on the peptide-specific immune response, which was low.

PF Publication Year (2):
2001

PF Publication Year (3):
2001

PF Publication Year (4):
2001

PF Publication Year (5):
2002

PF Publication Year (6):
2002

PF Publication Year (7):
2002

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L19: Entry 66 of 192 File: DWPI Jul 4, 2001

DERWENT-ACC-NO: 2001-542314
DERWENT-WEEK: 200161

TITLE: Adjuvant composition for vaccine

Basic Abstract Text (1):
NOVELTY - The present invention provides a novel adjuvant composite, containing liposome and CpG ODN, for both preventive and curative vaccines. The adjuvant composite has strong induced Th1 type and Th2 type responses and obvious synergistic effect. In addition, the vaccine adopting the adjuvant administration as well as nasal mucosa administration to induce general immunity response including body fluid immunity and cell immunity.

PF Publication Year (1):
2001

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L19: Entry 67 of 192 File: DWPI Jun 28, 2001

DERWENT-ACC-NO: 2001-475812
DERWENT-WEEK: 200151

TITLE: Reducing risk of anaphylactic hypersensitivity response to an allergen in a subject, by administering an immunomodulating nucleic acid molecule comprising a specific sequence

Basic Abstract Text (4):
The efficacy of beta -gal/immunostimulatory sequence (ISS-ODN) (5'-TGACTGTGAACGTTCGAGATGA-3') vaccination in protecting against the development of anaphylactic hypersensitivity was evaluated. C3H/HeJ mice received intradermal (i.d.) vaccinations with ISS-ODN (10 micro g) and beta -gal (10 micro g), ISS-ODN or beta -gal alone or beta -gal plus mutated oligodeoxynucleotide (M-ODN: 5'-TGACTGTGAACCTTCCAGATGA-3') (10 micro g) on 3 occasions 10 days apart. Twenty five days after the last vaccination, mice were Th2 sensitized with a mixture of beta -gal (100 micro g), pertussis toxin (300 ng), and alum (1 mg) injected intraperitoneally (i.p.) on 2 occasions 7 days apart. Mice then received an intravenous (i.v.) challenge with 150 micro g of beta -gal 3 weeks after their last i.p. sensitization. Plasma histamine levels were determined 2 minutes after challenge. p values for survival were determined by Fisher's exact test and p values for histamine release were determined by unpaired 2 tailed Student's test. Surviving mice demonstrated signs of anaphylaxis within the first half hour after challenge, but appeared healthy 1 hour later. The results showed that 5/12 beta -gal/ISS-ODN vaccinated mice survived i.v. beta -gal challenge versus 0/36 control mice receiving beta -gal or ISS/ODN or beta -gal and M-ODN. Post challenge histamine levels were also significantly lower in beta -gal/ISS-ODN vaccinated versus control mice. Those immunized with beta -gal and ISS-ODN demonstrated a 90% decrease in serum IgE post sensitization when compared to controls. In addition, while beta -gal/ISS-ODN vaccinated mice had similar IgG1 levels to control mice, IgG2a levels were 8-fold higher. Splenocytes from beta -gal/ISS-ODN vs. control vaccinated mice demonstrated an antigen specific Th1 cytokine bias reflected in elevated IFN gamma and depressed IL-4 and IL-5 production upon culture with allergen.

PF Publication Year (1):
2001

PF Publication Year (2):
2001

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L19: Entry 69 of 192 File: DWPI Feb 27, 2001

DERWENT-ACC-NO: 2001-217934
DERWENT-WEEK: 200535

TITLE: Immunostimulatory composition useful for stimulating immune response in a subject, comprises antigen and immunostimulatory nucleic acid comprising oligonucleotides having unmethylated cytosine-guanine dinucleotides

Basic Abstract Text (10):
To determine whether CpG ODN can cause in vivo immune stimulation, DBA/2 mice were injected once intraperitoneally with phosphate buffered saline (PBS) or phosphorothioate CpG or non-CpG ODN at a dose of 33 mg/kg. Spleen cells from mice were examined 24 hours after ODN injection for expression of B cells surface activation markers Ly-6A/E, Bla-I and class II MHC using three color flow cytometry and for their spontaneous proliferation using 3H thymidine. Expression of all three activation markers was significantly increased in B cells from mice injected with CpG ODN, but not from mice injected with PBS or non-CpG ODN. Spontaneous 3H thymidine incorporation was increased by 2-6 fold in spleen cells from mice injected with the stimulatory ODN compared to PBS or non-CpG ODN-injected mice.

Basic Abstract Text (11):
USE - (I) is useful for enhancing an immune response in a subject, by administering (I) which enables antigen-specific B-cell activation (claimed). Immunostimulatory ODN compositions are useful for activating lymphocytes in a subject and for treating, preventing or ameliorating an immune system deficiency e.g. tumor or cancer or viral, fungal, bacterial or parasitic infection in a subject. The immunostimulatory nucleotides can be administered as a vaccine to stimulate a subject's response to a vaccine and for treating leukemia by increasing the sensitivity of chronic leukemia cells to subsequent chemotherapy.

PF Publication Year (1):
2001

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L19: Entry 71 of 192 File: DWPI Jun 26, 2003

DERWENT-ACC-NO: 2001-138066
DERWENT-WEEK: 200343

TITLE: Enhancing immune response against pathogen or antigen associated with infectious diseases, an allergen or cancer, involves administering immunostimulatory nucleotide sequence prior to antigen exposure

Basic Abstract Text (1):
NOVELTY - Enhancing an immune response to a substance, immunizing a subject against a substance, or eliciting immunoglobulin (Ig)G2 antibody production, comprising administering an immunostimulatory nucleotide sequence (ISS) to a subject at least one hour prior to exposure to the substance, is new.

PF Publication Year (2):
2001

PF Publication Year (3):
2001

PF Publication Year (4):
2002

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L19: Entry 72 of 192 File: DWPI Mar 27, 2003

DERWENT-ACC-NO: 2001-006880
DERWENT-WEEK: 200325

TITLE: Novel oligonucleotides useful for the prevention and treatment of allergies, cancer, and autoimmune disorders and for ameliorating symptoms resulting from exposure to a bio-warfare agent

Basic Abstract Text (12):
MECHANISM OF ACTION - Inducer of cell or humoral mediated immune response. Vaccine. Oligodeoxynucleotides (ODNs) ATCGACTCTCGAGCGTTCT and ATCGACTAGCGTTCGTTCTC were synthesized on a DNA synthesizer and the induction of immune response by the ODNs was tested. The normal DNA backbone phosphodiesterase linkages were replaced with phosphorothioate linkages. Human peripheral blood mononuclear cells were incubated for 72 hours with the ODNs. Interleukin (IL-6) and tumor necrosis factor gamma (TNF- gamma) levels were determined by enzyme linked immunosorbent assay (ELISA) using anti-IL-6 and anti-TNF- gamma antibodies. Cell proliferation was determined by (3H) thymidine incorporation. The results showed that the sequence containing 5'-N1N2N3T-CpG-WN4N5N6 3' was desirable to induce a humoral immune response. In addition, maximum induction was observed for ODNs that contained a phosphorothioate backbone. The sequence containing 5'RY-CpG-RY 3', was desirable to induce a cell-mediated immune response. The IL-6 levels and cell proliferation obtained with ATCGACTCTCGAGCGTTCT was 85 and 44 and the IL-6 level obtained with ATCGACTAGCGTTCGTTCTC was 55.

PF Publication Year (2):
2000

PF Publication Year (3):
2000

PF Publication Year (4):
2002

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